(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.93 Tetracycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride diagnostic sensitivity powder is crystalline tetracycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to tetracycline. Each vial contains 20 milligrams of tetracycline hydrochloride. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.8 and not more than 3.0. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1) (i), (vi), and (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of tetracycline hydrochloride in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, moisture, pH, crystallinity, and absorptivity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

Subpart C—Susceptibility Test Panels

SOURCE: 43 FR 9793, Mar. 10, 1978, unless otherwise noted.

§ 460.100 Antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Antimicrobial susceptibility test panels are polystyrene trays molded with separate wells which contain frozen aliquots of antibiotic and non-antibiotic antimicrobial solutions in Mueller-Hinton broth. The trays are used in clinical laboratories for determining susceptibility of microorganisms to antimicrobial drugs. The broth antimicrobial solutions are prepared from serially diluted antimicrobial stock solutions. The concentrated aqueous antimicrobial stock solutions must conform to the requirements for certification prescribed by this section.

(2) *Labeling.* In addition to the requirements of §§ 432.5 and 809.10 of this chapter, each test panel shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or immediate container of trays:

(*a*) The batch mark.

(*b*) The name and potency of each solution in the batch according to the following:

Name of drug	Content of antimicrobic in micrograms per milliliter
Ampicillin (gram-positive panel)	8, 4, 2, 1, 0.5, 0.25, 0.12.
Ampicillin (gram-negative panel)	16, 8, 4, 2, 1, 0.5, 0.25.
Carbenicillin	512, 256, 128, 64, 32, 16, 8.
Cephalothin	64, 32, 16, 8, 4, 2, 1.
Chloramphenicol	32, 16, 8, 4, 2, 1, 0.5.
Clindamycin	16, 8, 4, 2, 1, 0.5, 0.25.
Colistin	4.
Erythromycin	16, 8, 4, 2, 1, 0.5, 0.25.
Gentamicin	16, 8, 4, 2, 1, 0.5, 0.25.
Kanamycin	64, 32, 16, 8, 4, 2, 1.
Methicillin	16, 8, 4, 2, 1, 0.5, 0.25.
Penicillin G	4, 2, 1, 0.5, 0.25, 0.12, 0.06.
Tetracycline	16, 8, 4, 2, 1, 0.5, 0.25.
Tobramycin	16, 8, 4, 2, 1, 0.5, 0.25.
Nitrofurantoin	64.
Trimethoprim (Gram-positive and gram-negative panel).	32, 16, 8, 4, 2, 1, 0.5.
Trimethoprim (Combination identification panel).	2.
Sulfamethoxazole (Gram-positive and gram-negative panel).	608, 304, 152, 76, 38, 19, 9.5.
Sulfamethoxazole (Combination identification panel).	38.

(*c*) The statement ''For in vitro diagnostic use''.

(ii) On each tray: The name of the panel, the expiration date, and the batch mark, including filling operation identification.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The concentrated antimicrobial stock solutions used in making the batch for potency and pH.

(*b*) The antimicrobial susceptibility test panels from the batch for performance and identity.

(ii) Samples required: (*a*) The concentrated antimicrobial stock solutions used in making the batch as directed in each individual monograph.

(*b*) The batch: A minimum of 25 panels selected at such intervals throughout the entire time of the filling operation so that the quantities of panels filled during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Performance*—(i) *Procedure.* Test randomly selected panels with each of the four test organisms as follows: Use the test organism suspensions prepared as described in § 460.6(b) (14), (15), (16), and (17). Transfer 0.005 milliliter of the appropriate test organism suspension into all wells of a panel. For the purpose of this section, wells are identified 1–10 from left to right and A–H from front to rear. Incubate inoculated panels in groups of three or less, each with a clean cover, at 35° C for 16 to 18 hours. For each control organism, the lowest concentration showing complete inhibition of growth is the minimal inhibitory concentration for that particular antimicrobial agent (referred to hereafter as its end point). For the purpose of this section, an on-scale end point means an end point which has been established by growth in the next lower concentration well. No growth in any well in row G does not indicate an on-scale end point unless the next lower concentration can be shown to produce growth. Establishment of an end point for a no-growth result in a well in row G requires additional testing in which 0.1 milliliter of sterile medium N is added to well G in some panels prior to inoculation with the test organism. An on-scale end point in row

G is established by no growth in the undiluted well G and growth in the two-fold diluted well G.

(ii) *Evaluation.* Susceptibility test panels of each filling operation pass the performance test if the on-scale end point for each antimicrobial agent and each test organism meet the limits specified in the following table (allowable off-scale end points are identified by asterisks):

PERFORMANCE END POINT ACCEPTANCE LIMITS

Antimicrobic	END POINTS (MICROGRAMS PER MILLILITER)			
	E. coli (ATCC 25922)	*S. faecalis* (ATCC 29212)	*S. aureus* (ATCC 29213)	*P. aeruginosa* (ATCC 27853)
Ampicillin	1–4	0.5–2	0.25–1	Greater than 8 (gram-positive panel). Greater than 16 (gram-negative panel).
Carbenicillin	4*–16	16–64	No growth	16–64.
Cephalothin	4–16	16–64	...do	64-greater than 64.
Chloramphenicol	2–8	4–16	4–16	Greater than 32.
Clindamycin	16-greater than 16.	4–16	No growth	Greater than 16.
Colistin	No growth	Growth	Growth	No growth.
Erythromycin	16-greater than 16.	1–4	0.125*–0.5	Greater than 16.
Gentamicin	0.125*–0.5	4–16	0.125*–0.5	0.125*–0.5
Kanamycin	1–4	16–64	0.5*–2	Greater than 64.
Methicillin	Greater than 16.	Greater than 16.	0.5–2	Greater than 16.
Nitrofurantoin	No growth	No growth	No growth	Growth.
Penicillin G	Greater than 4.	1–4	0.125–0.5	Greater than 4.
Tetracycline	0.25–1	8-greater than 16.	0.125*–0.5	4–16.
Trimethoprim-sulfamethoxazole.	No growth	No growth	No growth	16/304-greater than 32/608 (gram-positive and gram-negative panel).
	...do	...do	...do	Growth (combination identification panel).
Tobramycin	0.25–1	8-greater than 16.	0.125*–0.5	No growth.

*An additional two-fold dilution below well G.

(2) *Identity*—(i) *Test procedure.* Test a randomly selected panel. Use the test organism suspensions prepared as described in § 460.6(b) (18), (19), and (20), and use one panel for several organisms as shown in the following table:

Test organism			Identity test data			
Name	ATCC No.	Suspension No.	Antibiotics tested	Type of panel and well to be inoculated		Response
				Gram-positive	Gram-negative	
S. aureus	29247	18	Methicillin	3A		No growth.
			Penicillin	4A		Growth.
			Erythromycin	2E		Do.
			Cephalothin	6D	2D	No growth.
			Clindamycin	1E	4D	Do.
			Tetracycline	8D		Growth.
Ent. cloacae	29249	19	Cephalothin		2B	Do.
			Kanamycin		7B	No growth.
Ps. aeruginosa	29248	20	Gentamicin		3D	Growth.
			Tobramycin		8D	No growth.

(Rows are numbered 1–10 from left to right; A–H from front to rear.)

Transfer 0.005 milliliter of the test organism suspension into designated wells of the panel. Incubate the panels at 35° C for 16 to 18 hours in covered stacks of three or fewer panels. Read the designated wells for growth or no growth.

(ii) *Evaluation.* Each susceptibility test panel passes the identity test if the determinations of growth in the designated wells agree with those shown in the table in paragraph (b)(2)(i) of this section.

[43 FR 9793, Mar. 10, 1978; 43 FR 12859, Mar. 28, 1978]

§ 460.110 Ampicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Ampicillin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous ampicillin trihydrate stock solutions serially diluted with distilled water to contain approximately 3,200, 1,600, 800, 400, 200, 100, and 50 micrograms ampicillin per milliliter. The potency of each solution is satisfactory if it is not less than 100 percent and not more than 150 percent of the number of micrograms of ampicillin that it is represented to contain. The pH of the solution containing 3,200 micrograms of ampicillin per milliliter is not less than 4.0 and not more than 7.0. The ampicillin trihydrate used conforms to the requirements of § 440.7(a)(1) (i), (iii), (iv), (v), (vi), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 2.5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 3,200 micrograms of ampicillin per milliliter.

§ 460.113 Carbenicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Carbenicillin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous carbenicillin disodium stock solutions serially diluted with distilled water to contain approximately the following concentrations: 20,480, 10,240, 5,120, 2,560, 1,280, 640, and 320 micrograms of carbenicillin per milliliter. The potency of each solution is satisfactory if it is not less than 100 percent and not more than 150 percent of the number of micrograms of carbenicillin that it is represented to contain. The pH of the solution containing 20,480 micrograms of carbenicillin per milliliter is not less than 6.0 and not more than 8.0. The carbenicillin disodium used conforms to the requirements of § 440.13a (a)(1) (i), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 20 micrograms of carbenicillin per milliliter (estimated).